Development of the metagenome-based methodology for monitoring deep-sea microbial and meiofaunal communities

Principal Investigator: Koji HAMASAKI, Michinari SUNAMURA, Kazutoshi YOSHITAKE, Hideto TAKAMI,Shigeaki KOJIMA, Chuya SHINZATO, Yuki MINEGISHI, Motohiro SHIMANAGA
Institution: The University of Tokyo, Kashiwa City, Kashiwa, JAPAN
Tel: 04-7136-6171 / Fax: 04-7136-6171
E-mail: hamasaki@aori.u-tokyo.ac.jp
Cooperated by: Kumamoto University

[Abstract]

Key Words: Deep-sea, Sediment, Metagenomics, Prokaryote, Meiobenthos, Species diversity

The purpose of this study is to develop the metagenome-based methodology that can be applied to the communities of micrometer-size organisms in deep-sea sediment samples, as part of the development of low-cost and efficient methods for continuous multipoint biodiversity monitoring in offshore seafloor (deep-sea) marine protected areas.

In subtheme 1, we developed prokaryotic diversity assessment protocols for deep-sea reserves using 16SrRNA gene amplicon sequencing and metagenome shotgun sequencing in order to enable the acquisition of comprehensive species diversity and functional gene information. A comparison and evaluation were performed using sediment and water samples collected from seamount areas during several cruises. We also evaluated the method to reconstruct genome information of unculturable species from metagenome sequence reads, constructed the genomes of 89 individual species and identified a novel bacterial species of the family Methylomirabilaceae as a characteristic dominant species in the protected area seamount sediments that rarely exists elsewhere. In order to characterize prokaryotic community in terms of functional diversity, a protocol for functional gene analysis based on the completion ratio of KEGG modules and their abundance using the Genomaple system was developed.

In subtheme 2, we established methods to process on board and preserve bottom-sediment samples collected by core samplers for metagenomic and environmental DNA analyses of deep-sea meiobenthic organisms. We further established methods to extract DNAs of meiobenthos from sediment samples and obtain their metagenomic data efficiently. We provided dataset of the DNA barcode (the Folmer region within mitochondrial COI gene) to construct molecular phylogenetic trees of the second dominant deep-sea meibenthos, harpacticoides as a model, and showed their species diversity and a number of individuals of each species within sediment samples can be estimated based on a molecular phylogenetic tree re-constructed using metagenomic data. By comparison with results of traditional morphological classification, the variability of the present methods was confirmed. We also established methods to obtain PCR products of metazoan DNA barcodes from environmental DNA within bottom-sediment samples. The present results of the molecular classification can be used as with those by the morphological classification and it can apply larva and broken individuals as well.

Overall, the methodologies and protocols we developed here are useful to obtain diversity information required for the EBSA criteria such as species richness, evenness, uniqueness, rarity and productivity of dee-sea benthic prokaryotes and meiofauna, which can contribute for routine monitoring of benthic organisms in offshore deep-sea marine protected areas.